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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte GEORGE NELSON BENNETT and MARY LOU HARRISON[1]

Appeal 2008-6009
Application 10/699,511
Technology Center 1600

Decided: [2] May 19, 2009

Before JAMES T. MOORE, *Vice Chief Administrative Patent Judge*, and RICHARD E. SCHAFER and SALLY G. LANE, *Administrative Patent Judges*.

MOORE, *Vice Chief Administrative Patent Judge*.

DECISION ON APPEAL

STATEMENT OF THE CASE

The Appellants appeal under 35 U.S.C. § 134 (2002) from a final rejection of claims 1-7. We have jurisdiction under 35 U.S.C. § 6(b) (2002).

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[1] The real party in interest is Rice University. (App. Br. 3).

[2] The two-month time period for filing an appeal or commencing a civil action, as recited in 37 CFR § 1.304, begins to run from the decided date shown on this page of the decision. The time period does not run from Mail Date (paper delivery) or Notification Date (electronic delivery).

The Appellants' claims are directed to a method for assembling polymerase chain reaction ("PCR") fragments of DNA "into an ordered circular arrangement for replication and genetic work in cells." (Specification p. 1, ll. 11-12). The method is said to "not rely on restriction enzymes." (Id., p. 2).

According to the Appellants, "[c]urrent methods of manipulating DNA fragments are each limited by size." (Id. p. 1, l. 17). Moreover, use of restriction sites is said to be problematic in that the orientation of the resulting fragment is not defined (Id. p. 2, l. 3) and "many restriction enzymes also cleave *within* a large PCR fragment ...." (Id. p. 2, l. 4)(emphasis added).

Claim 1 is the only independent claim in the application and the Appellants do not argue any claims or rejections separately. Therefore, we select independent claim 1 to decide the appeal. See 37 C.F.R. § 41.37(c)(1)(vii)(2006). Accordingly, the remaining claims stand or fall with claim 1.

Claim 1 reads as follows:

> 1. A method of assembling PCR fragments, comprising;
>
> a) making a first PCR fragment with first and second primers, wherein the second primer comprises a modified nucleotide that can be removed by a DNA repair enzyme, resulting in a 3′ overhang, and wherein the first PCR fragment comprises a first site specific recombinase site;
>
> b) treating the first PCR fragment with a DNA repair enzyme to generate a 3′ overhang and immobilizing the first PCR fragment on a solid support or vice versa;

c) making a second PCR fragment with third and fourth primers, wherein the third and fourth primers each comprises a modified nucleotide that can be removed by a DNA repair enzyme resulting in a 3′ overhang;

d) treating the second PCR fragment with a DNA repair enzyme to generate a 3′ overhang;

e) annealing and ligating the first and second PCR fragments;

f) optionally repeating steps c, d and e until a last PCR fragment is added to the growing chain to produce an assembled fragment, wherein the last PCR fragment comprises a second site specific recombinase site; and

g) simultaneously removing and circularizing the assembled fragment from the solid support with a site specific recombinase in a single step.

(Additional indentation added, see 37 CFR §1.75(i)).

THE EXAMINER'S EVIDENCE

The Examiner relies upon the following as evidence in support of the rejections:

Elledge	5,851,808	Dec. 22, 1998

Watson, et al. "Cloning and Assembly of PCR Products Using Modified Primers and DNA Repair Enzymes" BioTechniques, vol. 23, no. 5 (1997), pp. 858-862.

Stahl et al., "Solid-Phase Gene Assembly of Constructs Derived from the Plasmodium falciparum Malaria Blood-Stage Antigen Ag332" Biotechniques, vol. 14, no. 3 (1993), pp. 424-436.

THE APPELLANTS' EVIDENCE

The Appellants further rely upon the following additional evidence in support of the appeal:

Declaration of Dr. George N. Bennett, dated May 17, 2007.

Kilbride E.A., et al., *Determinants of product topology in a hybrid Cre-Tn3 resolvase site specific recombination system*, J Mol Biol. 355(2): 185-95 (2006).

Vetcher A.A., et al., *DNA topology and geometry in Fly and Cre recombination*, J Mol Biol. 357(4): 1089-1 04 (2006).

Grainge I., et al., *Symmetric DNA sites are functionally asymmetric within Flp and Cre site specific DNA recombination synapses*, J Mol Biol. 320(3): 515-27 (2002).

Crisona N.J., et al., *The topological mechanism of phage lambda integrase*, J Mol Biol. 18; 289(4):747- 75 (1999).

Kilbride E., et al., *Topological selectivity of a hybrid site-specific recombination system with elements from Tn3 res/resolvase and bacteriophage P1 loxP1Cre*. J Mol Biol. 289(5):1219-30 (1999).

Advisory Action, October 16, 2007.

THE REJECTION

The following rejection is before us for review:

Claims 1-7 stand rejected under 35 U.S.C. § 103(a) over the combination of Watson, Elledge and Stahl.

We REVERSE.

ISSUE

Have the Appellants established that the Examiner erred in determining that it would have been obvious to one of ordinary skill in the art at the time the invention was made to assemble PCR fragments on a solid support using a site specific recombinase?

FINDINGS OF FACT

The record supports the following findings of fact by a preponderance of the evidence.

1. Watson describes a method of assembling PCR fragments, comprising preparing a first fragment with two primers, where one primer comprises a modified nucleotide that can be removed by a DNA repair enzyme to create a ligatable 3′ overhang. (Watson Abstract, p. 858).

2. Watson also describes treating the first PCR fragment with a DNA repair enzyme to generate a 3′ overhang. (Id.).

3. Watson therefore describes step (a) of the instant claim.

4. Watson also describes step (b) of the instant claim with the exception of immobilization of the first fragment on a solid support.

5. Watson describes preparing a second PCR fragment with two primers that each comprise a modified nucleotide that can be removed by a DNA repair enzyme resulting in a 3′ overhang. (Id.).

6. Watson also describes treating the second PCR fragment with a DNA repair enzyme to generate a 3′ overhang. (Id.).

7. Watson therefore describes steps (c) and (d) of the instant claims.

8. Watson next describes annealing and ligating the PCR fragments consecutively, i.e., an "ordered joining of PCR fragments to make functional DNA assemblies." (Id.).

9. Watson therefore describes step (e) of the instant claims.

10. Watson describes repeating the steps of preparing a PCR fragment with two primers each having modified nucleotides that can be removed by a DNA repair enzyme to generate 3′ overhangs and then performing the ordered joining of the PCR fragments, until a last PCR fragment is added to the growing chain to produce an assembled fragment. (Id.).

11. Watson therefore describes step (f) of the instant claims, with the exception of a second site specific recombinase site.

12. Watson describes circularizing the assembled fragment via ligation into a vector. (Id. at 860).

13. Watson therefore describes step (g) of the instant claim, with the exception of removing the assembled fragment from the solid support.

14. The differences between the instant claim 1 and Watson are that:

- Watson does not describe immobilizing the first PCR fragment on a solid support, and

- Watson also does not describe simultaneously removing and circularizing the assembled fragment from a solid support with a site specific recombinase.

15. Elledge describes a method of rapidly subcloning nucleic acid sequences "without the need to use restriction enzymes." (Elledge 1:55-58).

16. Elledge describes using a site-specific recombinase, i.e., the *Cre/lox* system, to catalyze the fusion/recombination (circularization) of

DNA in relation to site-specific recombinase target sites in solution. (Id. 14:45-15:28).

17. Stahl describes assembling gene fragments on a solid support as an alternative to assembly in solution. (Stahl p. 424).

18. Stahl describes that immobilizing gene fragments on a solid support offers convenience, efficiency, and the ability to manufacture extended fragments in a directed manner. (Id. at 432).

19. Stahl describes removing the assembled fragment from the solid support prior to subcloning. (Id. at 426).

20. According to the Examiner, Stahl describes using restriction enzymes and ligases on the solid support.

ANALYSIS

I. The Examiner's Rejection

Claims 1-7 stand rejected under 35 U.S.C. § 103(a) over the combination of Watson, Elledge and Stahl. Specifically, the Examiner found that Watson describes a method of assembling PCR fragments comprising each of the steps set forth in Appellants' claim 1, except that Watson does not teach (1) using site specific recombination, as recited in step(a); (2) immobilizing the first PCR fragment on a solid support, as recited in step (b); and (3) simultaneously circularizing and removing the assembled fragment from a solid support with a site specific recombinase, as recited in step (g). (Final Rejection, June 26, 2007, 3-4).

However, the Examiner found that Elledge describes "site specific recombination and circularization occurring simultaneously in a single step, with recombinase." (Id. at 4). In particular, the Examiner found that

Elledge teaches site specific recombination with Cre recombinase in vitro. According to the Examiner, "By employing the Cre/lox system for recombination of two plasmids, Elledge necessarily teaches simultaneous circularization and recombination of the plasmid." (Id.).

The Examiner found that one of ordinary skill in the art at the time of the invention would have been motivated to apply Elledge's method of using recombinase to combine DNA with Watson's method of DNA assembly "to reduce the time and effort associated with restriction mediated DNA assembly." (Id.). According to the Examiner, Elledge teaches that "site specific recombination eliminates the use of restriction enzymes and DNA ligase," and instead requires only a single recombinase enzyme. (Id.).

Additionally, the Examiner found that Stahl teaches immobilizing PCR fragments for assembly. (Id. p. 5)(citing Stahl p. 424 Abstract and p. 425, Fig. 1). The Examiner found that Stahl teaches that "[i]mmobilization of the first oligonucleotide enables controlled stepwise annealing/ligation of successive 5′ phosphorylated oligonucleotides to rapidly build up accurate gene constructs making it possible to subclone for subsequent expression of the gene product." (Id.)(citing Stahl p. 424, col. 3). The Examiner also found that Stahl teaches subsequently removing the assembled gene construct from the solid support prior to subcloning. (Id.)(citing Stahl p. 426, col. 2).

According to the Examiner, a skilled artisan at the time of the invention would have been motivated to apply Stahl's step of immobilizing the fragments for assembly in the combination "to have a controlled assembly of the fragments." (Id.). Similarly, the Examiner concluded that it

would have been obvious to the skilled artisan to apply Stahl's immobilization step in the combination "to stabilize and control the assembly of the gene constructs," as a "[c]ontrolled assembly yields more accurate gene constructs." (Id.).

II. The Appellants' Contentions

The Appellants contend that the obviousness rejection cannot be maintained because that the Examiner has not established a reasonable expectation of success for using recombinase on a solid support. (App. Br. 13). According to the Appellants, the "Examiner merely assumes that the recombinase method of Elledge *can* be applied to the solid support method of Watson with a reasonable expectation of success" because *Cre/lox* is a recombinase known to recombine and circularize plasmid DNA. (Id. at 14). The Appellants assert that the Examiner's basis for an expectation of success is "merely conclusory" and lacks "any rationale to support an assertion of reasonable expectation of success." (Id. 13-14)(citing *KSR Int'l Co. v. Teleflex, Inc* , 550 U.S. 398, 419). In particular, the Appellants challenge the Examiner's finding of a reasonable expectation of success by asserting that because recombinases are topologically sensitive, the recombinases "are always used in solution (or cells) where the DNA molecules can freely move around to assume the complex knotted forms required during the recombinase reaction." (App. Br. 15-16). According to the Appellants, "[i]t is for this reason that Watson did not think to apply recombinases to his method." (Id. p. 16)(citing Bennett Declaration). The Appellants support this contention by referencing the declaration of inventor Dr. George N. Bennett. Dr. Bennett's declaration "shows that topologically

sensitive recombinases are not expected to function on substrates tethered to a solid support." (App. Br. 16).

Under the specific facts of this case, we agree with the Appellants. Presently, the Examiner has not established a reasonable expectation of success for using recombinase method of Elledge on a solid support. The Examiner reasoned that Stahl teaches "that restriction enzymes and ligases can be successfully used with DNA constructs immobilized on a solid support." (Answer p. 10). The Examiner explained that similar to recombinases, restriction enzymes and ligases are also "topologically sensitive in that both of these enzyme have site (sequence) specific requirements and the specific sequence must be accessible to the enzymes for them to function properly." (Id.). According to the Examiner, Stahl ensured that when the DNA was immobilized to the support that the specific sites required by the enzyme to function were available and accessible to the enzyme." (Id.).

Thus, the Examiner determined that Stahl demonstrates that immobilizing DNA to a solid support does not significantly alter the structure of DNA such that enzymes requiring specific sequences and accessibility to those sequences are inhibited or impossible. (Id.). According to the Examiner, a skilled artisan who reviewed Stahl would have recognized that this principle would similarly apply to the use of the Cre recombinase enzyme such that the artisan would have a reasonable expectation of success for its use on a solid support also. (Id.).

While Stahl describes that restriction enzymes and ligases function effectively with DNA immobilized on a solid support, we do not find that

the reference describes that these enzymes and ligases are topologically sensitive.

The Examiner has not provided other evidence to support its rationale that Stahl's teaching relating to the usefulness of restriction enzymes and ligases would similarly apply to the use of the Cre recombinase enzyme such that the artisan would have a reasonable expectation of success for its use on a solid support.

Consequently, we find that the Examiner has not shown that one of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success in making the combination as claimed.

Accordingly, we reverse the Examiner's rejections.

CONCLUSION OF LAW

On the record before us, the Appellants have established error on the part of the Examiner. The Examiner has not established that the claims are obvious over the combined prior art.

DECISION

The Rejection of claims 1-7 under 35 U.S.C. §103(a) as being unpatentable over the combination of Watson, Elledge and Stahl is REVERSED.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a)(1)(iv) (2006).

REVERSED

ack

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